DATA EVALUATION RECORD

TRIFLUMEZOPYRIM

STUDY TYPE: CARCINOGENICITY - MOUSE

(OCSPP 870.4200b)

MRID 49382174

Prepared for

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Task 6-169

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DATA EVALUATION RECORD[1]

STUDY TYPE: Carcinogenicity - Mouse;
OCSPP 870.4200b [§83-2b]; OECD 451.

PC CODE: 129210 **DP BARCODE:** D432127

TEST MATERIAL (PURITY): Triflumezopyrim technical (99.4% a.i.)

SYNONYMS: DPX RAB55; 2,4-Dioxo-1-(5-pyrimidinylmethyl)-3-(3-(trifluoromethyl)-phenyl)-2H-pyrido(1,2-a)pyrimidinium inner salt

CITATION: Papagiannis, C. (2015); Triflumezopyrim (DPX-RAB55) technical: Oncogenicity study 18-month feeding study in mice. DuPont Report No.: 34940. MPI Research, Mattawan, Michigan, USA. Report No. 125-163. Study date: December 7, 2015. MRID No. 49382174.

SPONSOR: E.I. du Pont de Nemours and Company, Wilmington, Delaware 19898

EXECUTIVE SUMMARY:

In an 18-month carcinogenicity feeding study (MRID 49382174), triflumezopyrim was administered to male and female Crl:CD1®(ICR) mice (60/sex/dose) at concentrations of 0, 200, 800, 2500, and 7000 ppm. The overall (Week 1-77) mean daily intakes in male mice were 0, 20.08, 84.49, 248.26, and 726.72 mg/kg bw/day. The mean daily intakes in female mice were 0, 21.82, 88.04, 283.27, and 809.75 mg/kg bw/day. Parameters evaluated included body weight, body weight gain, food consumption, food efficiency, clinical signs, hematology, ophthalmology, organ weights, and gross and microscopic pathology.

No treatment-related effects were seen on survival, clinical signs, body weight, food consumption, and clinical pathology parameters at any dose level. Absolute and relative liver weights were statistically higher in males at 2500 ppm and 7000 ppm (15.69% and 37.39%) and in females at 2500 ppm and 7000 ppm (15.91% and 38.51%). Enlarged spleens noted in females at 7000 ppm correlated with increased spleen weights at 7000 ppm (95.57%). Histopathology revealed increased incidences of hepatocellular hypertrophy in males at 2500 ppm and 7000 ppm (17% vs. 3% in the control). In the absence of other effects indicative of liver injury (e.g., alterations in relevant clinical chemistry parameters and histopathology), changes in liver weights and hypertrophy observed at 7000 ppm were considered to be non-adverse, adaptive responses to exposure to a xenobiotic.

[1] This DER was generated by modifying the study summary in a Tier II document (MRID 49382105).

Dietary administration of triflumezopyrim resulted in an increased incidence of hepatocellular adenomas in male mice at 7000 ppm (28% vs. 15% in controls). There was also an increased incidence of alveolar-bronchiolar carcinomas in female mice at 7000 ppm (10% vs. 3% in controls). A Pathology Work Group (PWG) was convened to evaluate the significance of the lung tumor findings (Appendix L of the study report).

The No-Observed-Adverse-Effect-Level (NOAEL) was 7000 ppm, the highest concentration tested (726.72 mg/kg/day in males and 809.75 mg/kg/day in females). A Lowest-Observed-Adverse-Effect (LOAEL) was not established.

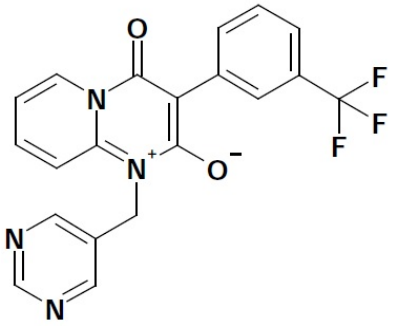
This carcinogenicity study in mice is classified **Acceptable/Guideline** and satisfies the guideline requirement for a carcinogenicity study [OCSPP 870.4200; OECD 451] in mice.

COMPLIANCE: Signed and dated GLP, Quality Assurance and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS:

A. MATERIALS:

1. Test material: Triflumezopyrim technical

Lot/Batch #:	RAB55-037
Purity:	99.4%
Description:	Neutral yellow powder
CAS #:	1263133-33-0
Stability of test compound:	The 17-day room temperature stability of the formulations from 100 to 20000 ppm in the diet had been established in a previously conducted study.
Structure:	

2. Vehicle: Untreated diet.

3. Test animals:

Species:	Mouse
Strain:	Crl:CD1®(ICR)
Age at dosing:	Approximately 52 days old
Weight at dosing:	25.9-36.5 g for males; 19.7-27.7 g for females
Source:	Charles River Laboratories, Inc., Portage, Michigan, USA
Acclimation period:	14 days
Diet:	PMI® Nutrition International, LLC Certified Rodent LabDiet® (#5002), *ad libitum*. During the test period, test substance was incorporated into the feed of all animals except negative controls.
Water:	Tap water, *ad libitum*
Housing:	Male animals were housed singly and females were pair-housed in solid bottom cages (polyboxes) with nonaromatic bedding.

4. Environmental conditions:

Temperature:	20–26°C
Humidity:	30–70%
Air changes:	Not reported
Photoperiod:	Alternating 12-hour light and dark cycles

B. STUDY DESIGN

1. In-life dates: Start: 08/23/2012; End: 02/27/2014

2. Animal assignment/dose levels: Five groups of 60 animals/sex/dose received triflumezopyrim in their diet at concentration of 0, 200, 800, 2500, and 7000 ppm daily for 18 months. These doses were equivalent to 0, 20.08, 84.49, 248.26, and 726.72 mg/kg bw/day in males and 0, 21.8, 88.04, 283.27 and 809.75 mg/kg bw/day in females, respectively. Animals were assigned to dose groups by computerized, stratified randomization so that there were no statistically significant differences among group body weight means within a sex. A negative control group received untreated diet.

Table 1. Dietary Carcinogenicity Study in Mice Fed Triflumezopyrim.

Test Group	Concentration in diet (ppm)[a]	Dose to animal (M/F) (mg/kg bw/day) [b]	No. of Animals (Males)	No. of Animals (Females)
1	0 (control)	0 / 0 (control)	60	60
2	200	20.08 / 21.8	60	60
3	800	84.49 / 88.04	60	60
4	2500	248.26 / 283.27	60	60
5	7000	726.72 / 809.75	60	60

Table taken from page 22 and values for animal doses were taken from section 4.2.5 of the study report (MRID 49382174).

3. **Dose Selection:** Doses were selected based on the results of a previous subchronic feeding study in mice in which no treatment-related effects were seen in male and female CD-1 mice fed diets containing 0, 200, 800, 250 and 7000 ppm (Limit Dose) for 90-days (MRID No. 49382160).

4. **Diet preparation and analysis:** The test substance was added to the rodent diet and thoroughly mixed for 30 minutes. Control diets were mixed for the same period of time. All diets were prepared every other week and kept at room temperature until used. The stability, homogeneity, and concentration of triflumezopyrim in the dietary mixtures were checked by analysis using HPLC at Weeks 1/2, 13/14, 25/26, 39/40, 51/52, 65/66, and 79/80. The test substance was at target concentrations ±20%, homogeneous (93.8 to 104.5%) throughout the feed, and was stable for up to 17 days at room temperature. Based on this information, it can be concluded that the animals received the targeted dietary concentrations of test substance during the study. No test article was found in the control diet.

Results:

Homogeneity analysis: 93.8 to 104.5% throughout the feed.

Stability analysis: The method validation study (MPI Research Study Number 125-160) established 17-day room temperature stability for the test diet.

Concentration analysis: the concentration analyses at 200, 800, 2500, and 7000 ppm were within the expected range of ± 20% of the nominal concentration at the analyzed intervals. The average percent of the nominal concentrations ranged between 93.8 and 104.5%.

5. **Statistics:**

Table 2. Statistical Analyses

Parameter	Preliminary test	If preliminary test is not significant	If preliminary test is significant
Body weight Body weight gain Food consumption Food efficiency Organ weight Differential leukocyte counts[a]	Levene's test for homogenicity	One-way analysis of variance followed by Dunnett's test	Welch's t-test with Bonferroni correction
Survival analysis	An overall comparison of groups was done using the log-rank test	Each treatment group was compared to the control group using a log-rank test.	
Tumor analysis	Cochran-Armitage test for trend	None	Fisher's Exact test was used to compare each treatment group with the control group.

			The survival adjusted test was conducted according to the prevalence/mortality methods described by Peto *et al.*
Non-tumor microscopic pathology data	Fisher's Exact test for independence	None	Fisher's Exact test was used to compare each treatment group with the control group

[a] A log transformation was performed on these data
[b] A rank transformation was applied followed with Dunnett's test.

Significance was judged at $p < 0.05$ and < 0.01. Separate analyses were performed on the data collected for each sex. The Reviewer considers the statistical analyses appropriate.

C. METHODS

1. **Observations:** Animals were observed at least once daily for mortality and morbidity and received detailed clinical examinations weekly to detect clinical signs of toxicity.

2. **Body weights:** All animals were weighed once per week during the first 13 weeks of the study and every other week thereafter.

3. **Food consumption and compound intake:** Food consumption was recorded for each animal over the weighing interval. For females, cage food consumption was measured and divided by the number of animals in the cage. Food efficiency and daily intake were calculated from food consumption and body weight data.

4. **Ophthalmoscopic examinations:** All animals were examined by focal illumination and indirect ophthalmoscopy prior to study start. All surviving animals were examined again prior to scheduled sacrifice.

5. **Hematology:** Blood samples were collected from all surviving animals at 12 and 18 months after initiation of the study. At sacrifice after 18 months of exposure, blood and bone marrow smears were prepared. The differential white blood cell count (neutrophils, band neutrophils, lymphocytes, monocytes, eosinophils, and basophils) was performed on all animals sacrificed *in extremis* and on the control and high dose animals at 18 months. The data did not indicate a need for the blood smears from the other dose groups and the 12-month interval to be examined.

Hematology parameters	
Neutrophils	Monocytes
Band Neutrophils	Eosinophils
Lymphocytes	Basophils

6. **Serological health screen:** A serological health screen for viruses was conducted pretest and at 6, 12, and 18 months on 2 to 5 randomly selected animals/sex using sentinel animals selected with a computerized randomization and euthanized *via* isoflurane anesthesia for this purpose.

7. **Plasma analysis:** An additional five groups of five animals/sex/concentration served as the toxiocokinetic (TK) group animals and received 0, 200, 800, 2500 and 7000 ppm through Week 52 for the purpose of blood collection. On test Day 364, blood was collected from these for possible analysis of concentrations of triflumezopyrim and/or

metabolites. Plasma was prepared and frozen but samples have not been analyzed. Data from these animals are not included in this summary.

8. **Sacrifice and pathology:** At termination, animals were sacrificed by carbon dioxide or isoflurane anesthesia and exsanguination. Gross examinations were performed on all main study animals. Organs that were weighed are listed in Table 3. Organ weight/final body weight and organ weight/brain weight ratios were calculated. Tissues collected from animals receiving the highest concentration (7000 ppm) and control (0 ppm) and from animals that died or were sacrificed prior to scheduled sacrifice were processed to slides and evaluated microscopically. Gross lesions and suspected target tissues (duodenum, glandular stomach, Harderian glands, liver, lung, spleen, and thymus for males and the glandular stomach, kidneys, liver, lung, mammary gland, spleen, thymus, and uterus with cervix for females) as determined by examination of the control and high dose animals, were processed to slides and examined microscopically for the 200, 800, and 2500 ppm animals.

Table 3. Carcinogenicity Study in Mice Fed Triflumezopyrim.

X	DIGESTIVE SYSTEM	X	CARDIOVASC./HEMAT.	X	NEUROLOGIC
	Tongue	X	Aorta, thoracic*	X	Brain (multiple sections)*+
X	Salivary glands*	X	Heart*+	X	Periph.nerve*
X	Esophagus*	X	Bone marrow*	X	Spinal cord (3 levels)*
X	Stomach*	X	Lymph nodes*	X	Pituitary*
X	Duodenum*	X	Spleen*+	X	Eyes (retina, optic nerve)*
X	Jejunum*	X	Thymus	X	GLANDULAR
X	Ileum*	X			Adrenal gland*+
X	Cecum*	X	UROGENITAL		Lacrimal gland
X	Colon*	X	Kidneys*+	X	Parathyroids*
X	Rectum*	X	Urinary bladder*	X	Thyroids*
X	Liver*+	X	Testes*+	X	OTHER
X	Gall bladder* (not rat)	X	Epididymides*+	X	Bone (sternum and/or femur)
X	Bile duct* (rat)	X	Prostate*	X	Skeletal muscle
X	Pancreas*	X	Seminal vesicle*	X	Skin*
X	RESPIRATORY	X	Ovaries*+	X	All gross lesions and masses*
X	Trachea*	X	Uterus*+	X	
X	Lung*++	X	Mammary gland*	X	
X	Nose*				
X	Pharynx*				
X	Larynx*				

* Required for carcinogenicity studies based on Guideline 870.4200.
+Organ weight required in carcinogenicity studies.
++Organ weight required if inhalation route.

II. RESULTS:

A. OBSERVATIONS:

1. **Clinical signs of toxicity:** There were no test substance-related clinical signs of toxicity observed.

2. **Mortality:** There were no test substance-related effects observed on survival in any group (Table 4).

Table 4. Survival rate

Test Group	Concentration in diet (ppm)[a]	Dose to animal (M/F) (mg/kg bw/day) [b]	No. of Animals (Males)	No. of Animals (Females)
1	0 (control)	0 / 0 (control)	52/60	48/60
2	200	20.08 / 21.8	53/60	44/60
3	800	84.49 / 88.04	50/60	48/60
4	2500	248.26 / 283.27	50/60	41//60
5	7000	726.72 / 809.75	44/60	42/60

Data taken from page 33 of the study report (MRID 49382174).

B. BODY WEIGHT AND BODY WEIGHT GAIN:

No treatment-related effects were seen on absolute body weights or body weight gains in male or female mice at any dose level (Tables 5 and 6).

Table 5. Body weights (g): carcinogenicity study in mice fed triflumezopyrim.

	0 ppm	200 ppm	800 ppm	2500 ppm	7000 ppm
Males					
	0 mg/kg/day	**20.08 mg/kg/day**	**84.49 mg/kg/day**	**248.26 mg/kg/day**	**726.72 mg/kg/day**
Week 13	40.57	41.12	41.71	41.12	40.25
Week 25	44.20	45.60	46.14	44.69	43.77
Week 51	45.92	47.65	48.20	46.63	45.84
Week 77	46.06	46.78	46.78	47.03	45.71
Females					
	0 mg/kg/day	**21.8 mg/kg/day**	**88.04 mg/kg/day**	**283.27 mg/kg/day**	**809.75 mg/kg/day**
Week 13	32.67	32.85	33.55	33.94	32.45
Week 25	37.25	35.22[b]	37.26	37.33	35.05[a]
Week 51	41.03	38.68	41.85	40.54	38.54[b]
Week 77	41.09	40.22	42.78	41.76	39.00

Data taken from page 35 of the study report (MRID 49382174).
[a] Significantly different from control by Welch's t-test criteria, $p < 0.05$
[b] Significantly different from control by Welch's t-test criteria, $p < 0.01$

Table 6. Body weight gain (g): carcinogenicity study in mice fed triflumezopyrim.

Parameter	0 ppm	200 ppm	800 ppm	2500 ppm	7000 ppm
Males					
	0 mg/kg/day	**20.08 mg/kg/day**	**84.49 mg/kg/day**	**248.26 mg/kg/day**	**726.72 mg/kg/day**
Body weight gain, Week 1-13	10.16	10.73	11.16	10.61	9.62
Body weight gain, Week 13-25	3.57	4.35	4.44	3.57	3.48
Body weight gain, Week 25-51	1.67	2.05	2.17	1.91	2.17
Body weight gain, Week 51-77	-0.11	-0.13	-1.44 [a]	-0.01	-0.06
Overall body weight gain	15.50	17.19	16.36	16.37	15.20
Females					
	0 mg/kg/day	**21.8 mg/kg/day**	**88.04 mg/kg/day**	**283.27 mg/kg/day**	**809.75 mg/kg/day**
Body weight gain, Week 1-13	9.07	9.75	9.96	10.29 [a]	8.75
Body weight gain, Week 13-25	4.59	2.37 [b]	3.71	3.36	2.60 [b]
Body weight gain, Week 25-51	3.73	4.02	4.30	3.30	3.49
Body weight gain, Week 51-77	0.42	1.34	0.89	1.29	0.73
Overall body weight gain	17.50	17.18	19.23	18.19	15.34

Data taken from page 35 of the study report (MRID 49382174).
[a] Significantly different from control by Welch's t-test criteria, $p < 0.05$
[b] Significantly different from control by Welch's t-test criteria, $p < 0.01$

C. FOOD CONSUMPTION, FOOD EFFICIENCY, AND DAILY INTAKE:

There were no treatment-related effects on food consumption or food efficiency in male or female mice at any dose level (Table 7).

Table 7. Food consumption and Food Efficiency: Carcinogenicity Study in Mice Fed Triflumezopyrim.

Parameter	0 ppm	200 ppm	800 ppm	2500 ppm	7000 ppm
Males (Food consumption, (g/animal/day)					
Dose (mg/kg/day)	**0**	**20.08**	**84.49**	**248.26**	**726.72**
Week 1–13	4.83	4.84	5.19[b]	4.85	5.01
Week 13–25	4.86	4.81	4.87	4.73	4.91
Week 25–51	4.68	4.83	5.00[b]	4.70	4.61
Week 51–77	4.65	4.60	4.68	4.54	4.63
Week 1–77	4.72	4.73	4.89	4.64	4.74
Food efficiency, (%) Week 1–13	2.33	2.49	2.39	2.42	2.10[a]
Food efficiency, (%) Week 13–25	0.81	0.99	1.00[a]	0.82	0.78
Food efficiency, (%) Week 25–51	0.19	0.22	0.23	0.23	0.25
Food efficiency, (%) Week 51–77	-0.01	-0.01	-0.18[a]	0.00	0.00
Food efficiency, (%) Week 1–77	0.61	0.67	0.62	0.65	0.60
Females (Food consumption, (g/animal/day)					
Dose (mg/kg/day)	**0**	**21.8**	**88.04**	**283.27**	**809.75**
Week 1–13	4.46	4.17[a]	4.36	4.42	4.18
Week 13–25	4.39	4.13	3.87	4.37	4.45
Week 25–51	4.64	4.48	4.67	4.62	4.45
Week 51–77	4.48	4.36	4.37	4.50	4.30
Week 1–77	4.59	4.42	4.60	4.59	4.42
Food efficiency, (%) Week 1–13	2.07	2.58[b]	2.25	2.56[b]	2.27
Food efficiency, (%) Week 13–25	1.55	0.72	0.84	0.84	0.60
Food efficiency, (%) Week 25–51	0.43	0.40	0.35	0.35	0.41
Food efficiency, (%) Week 51–77	-0.07	0.18	0.09	0.09	0.00
Food efficiency, (%) Week 1–77	0.70	0.73	0.71	0.71	0.62

Data taken from page 37 of the study report (MRID 49382174).
[a] Significantly different from control by Welch's t-test criteria, $p < 0.05$
[b] Significantly different from control by Welch's t-test criteria, $p < 0.01$

D. OPHTHALMOSCOPIC EXAMINATIONS:

No ophthalmological changes were seen at any dose level in either sex at any dose level.

E. CLINICAL PATHOLOGY:

1. Hematology:

No treatment-related changes were observed at any dose level.

2. Serological health screen:

All serological health screen results were negative for all the intervals.

F. SACRIFICE AND PATHOLOGY

1. **Organ weight:** As shown in Table 8 below, treatment-related organ weight increases were present in the liver of males at 7000 ppm and in females at 2500 and 7000 ppm, and in the spleen of females at 7000 ppm.

Table 8. Organ weights: Carcinogenicity Study in Mice Fed Triflumezopyrim.

Parameter	0 ppm	200 ppm	800 ppm	2500 ppm	7000 ppm
Males					
Dose (mg/kg/day)	**0**	**20.08**	**84.49**	**248.26**	**726.72**
Absolute liver with gall bladder weight (% control)	2.161±0.98	2.220±0.68 (+2.73)	2.247±0.64 (+3.98)	2.500±0.67 (+15.69)[b]	3.185±1.37 (+47.39)[b]
Relative[a] liver with gall bladder weight (% control)	5.0407±2.50	4.8888±1.24 (-3.01)	5.0794±1.55 (+0.77)	5.6363±1.23 (+11.82)	7.4689±3.18 (+48.17)[b]
Liver with gall bladder to brain weight (% control)	4.2283±1.71	4.4492±1.46 (+5.22)	4.4801±1.21 (+5.96)	4.9153±1.30 (+16.25)	6.3536±2.66 (+50.26)[b]
Females					
Dose (mg/kg/day)	**0**	**21.8**	**88.04**	**283.27**	**809.75**
Absolute liver with gall bladder weight (% control)	1.880±0.38	1.872±0.41 (-0.43)	1.938±0.41 (+3.09)	2.173±0.47 (+15.59[b])	2.604±0.51 (+38.51[b])
Relative[a] liver with gall bladder weight (% control)	4.8652±0.90	4.9029±0.96 (+0.77)	4.7691±0.80 (-1.98)	5.5928±1.18 (+14.96[b])	6.9829±1.15 (+43.53[b])
Liver with gall bladder to brain weight (% control)	3.6578±0.77	3.7036±0.88 (+1.25)	3.7546±0.80 (+2.65)	4.2547±1.00 (+16.32[b])	5.0727±1.01 (+38.68[b])
Absolute spleen weight (% control)	0.203±0.11	0.279±0.45 (+37.43)	0.195±0.11 (-3.94)	0.245±0.16 (+20.69)	0.397±0.27 (+95.57[b])
Relative[a] spleen weight (% control)	0.5340±0.31	0.7174±1.13 (+34.34)	0.4912±0.32 (-8.01)	0.6300±0.41 (+17.98)	1.0585±0.65 (+98.22[b])
Spleen to brain weight (% control)	0.3948±0.22	0.5718±1.01 (+44.83)	0.3780±0.21 (-4.55)	0.4821±0.32 (+22.11)	0.7698±0.50 (+94.98[b])

Data taken from pages 867-889 of the study report (MRID 49382174).
[a] Relative weight is defined as the organ to body weight ratio.
[b] Significantly different from control by Levine's test criteria, $p < 0.01$

2. **Gross pathology:** Treatment-related macroscopic changes were present in the liver in males at 7000 ppm, and in the spleen in females at 7000 ppm. A mass or nodule was noted in the liver of 27/60 males at 7000 ppm compared to 12/60 males from the control group. The masses and nodules generally correlated to hepatocellular adenomas. An enlarged spleen was present in 15/60 females at 7000 ppm compared to 5/60 females from the control group.

3. **Microscopic pathology:**

   a. **Non-neoplastic findings:** The non-neoplastic lesions observed in the liver, spleen, and mammary gland are presented in Table 9.

Table 9. Non-neoplastic Lesions: Carcinogenicity Study in Mice Fed Triflumezopyrim.

Triflumezopyrim (ppm)	**0**	**200**	**800**	**2500**	**7000**
Number of animals/group:	**60**	**60**	**60**	**60**	**60**
Males:					
Dose (mg/kg/day)	**0**	**20.08**	**84.49**	**248.26**	**726.72**
Liver					
Hypertrophy, hepatocyte, centrilobular	2[a] (3%)	3	3	7 (12%)	10 (17%)
Hematopoiesis, extramedullary	2	2	1	2	2
Spleen					
Hematopoiesis, extramedullary	46	48	46	52	53
Females:					
Dose (mg/kg/day)	**0**	**21.8**	**88.04**	**283.27**	**809.75**
Liver					
Hypertrophy, hepatocyte, centrilobular	0	0	0	1	1
Hematopoiesis, extramedullary	0	3	0	1	11[b] (18%)
Spleen					
Hematopoiesis, extramedullary	44	49	50	46	53
Mammary gland					
Dilatation, duct	7 (12%)	11	10	14	23[b] (38%)

Data taken from pages 890-1044 of the study report (MRID 49382174).
[a] Number of organs with microscopic change.
[b] Statistically significant by Fisher's exact test criteria, p <0.01

(i) **Liver:** There was a dose-related increase in the incidence of centrilobular hypertrophy in male mice at 2500 ppm (7/60; 12%) and 7000 (17%) ppm when compared to controls (2/60); 3%). The incidences of these lesions were very minimal (1/60) in female mice at these dose levels. In the absence of other effects (e.g. changes in liver enzymes and more severe histopathology like hyperplasia), this lesion generally is considered to be adaptive and not adverse.

(ii) **Spleen:** The increased spleen weights and the minor increase in the incidence of EMH in spleen were not associated with correlative changes indicative of adverse effects on red blood cell parameters (e.g., erythroid hyperplasia of bone marrow). Although red blood cell parameters were not evaluated in this study, no effects on these parameters were observed in a previous study in which mice were exposed to the test substance at concentrations up to 7000 ppm in the diet for 13 weeks (MRID 49382160).

(iii) **Mammary glands:** There was a statistically significant ($p<0.05$) increase in the incidences of mammary ductal dilatation at the high dose (23/60; 38%) when compared to controls (7/60; 12%). Mammary ductal dilatation is a spontaneous aging change seen in rodents (Rudman et al., 2012), and the increase in incidence in this study was only seen for lesions of minimal severity, and was not associated with cellular necrosis or degeneration, nor was there evidence of progression to a neoplastic lesion or cellular dysplastic change that would suggest a premalignant lesion.

b. **Neoplastic findings**: The neoplastic lesions observed in the liver and spleen are presented in Tables 10 and 11, respectively.

(i) Liver:

Table 10. Neoplastic Lesions in Male Mice: Carcinogenicity Study in Mice Fed Triflumezopyrim.

Dose (ppm)	**0**	**200**	**800**	**2500**	**7000**
Dose (mg/kg/day)	**0**	**20.08**	**84.49**	**248.26**	**726.72**
Number of livers examined	60	60	60	60	60
Adenoma, hepatocellular, benign	9[a] (15%)	6	4	12 (20%)	17[b] (28%)
Carcinoma, hepatocellular, malignant	1	0	0	0	1

Data taken from pages 1045-1095 of the study report (MRID 49382174).
[a] Number of organs with microscopic change.
[b] Statistically significant **trend** only by Cochran-Armitage and Peto test criteria, $p < 0.05$

Increased incidences of hepatocellular adenomas were observed in male mice at 7000 ppm (28% vs. 15% in controls). Liver adenomas were characterized by well demarcated areas composed of well-differentiated proliferative hepatocytes with compression of the adjacent tissue. Neoplastic cells had altered staining properties and were either smaller or larger than the adjacent normal hepatocytes. Within the tumors, there was loss of the normal lobular architecture. In addition to the increased number of males with hepatocellular adenomas at this dose, affected males had an increased number of multiple adenomas in the liver as well. Such an increase of hepatocellular adenomas per affected animal was not seen at the 2500 ppm level.

(ii) Lung:

Table 11. Neoplastic Lesions in Female Mice: Carcinogenicity Study in Mice Fed Triflumezopyrim.

Dose (ppm)	**0**	**200**	**800**	**2500**	**7000**
Dose (mg/kg/day)	**0**	**21.8**	**88.04**	**283.27**	**809.75**
Number of lungs examined	60	60	60	60	60
Carcinoma, bronchiolar-alveolar, malignant	2[a] (3%)	0	1	4	6[b] (10%)

Data taken from pages 1045-1095 of the study report (MRID 49382174).
[a] Number of organs with microscopic change.
[b] Statistically significant trend only by Cochran-Armitage and Peto test criteria, $p < 0.05$

There was a slight increase in the incidence of bronchiolo-alveolar carcinomas in the lungs of female mice at 7000 ppm (809.75 mg/kg/day). No increased incidences of bronchiolo-alveolar hyperplasia or adenomas were seen. Bronchiolo-alveolar carcinomas were expansile masses containing pleomorphic tumor cells with occasional crowding of nuclei.

To further clarify the significance of this finding, a Pathology Working Group (PWG) was convened. PWG's consensus diagnosis for the proliferative lesions in the lung of female mice are presented in Table 12.

Table 12. PWG Diagnosis of Lung Lesions in Female Mice Fed Triflumezopyrim[a].

Dose (ppm)	**0**	**200**	**800**	**2500**	**7000**
Dose (mg/kg/day)	**0**	**21.8**	**88.04**	**283.27**	**809.75**
Number of lungs examined	60	60	60	60	60
Bronchiolar alveolar Hyperplasia	2	10	8	5	3
Bronchiolar alveolar Adenoma	6	8	6	9	10
Bronchiolar alveolar Carcinoma	2	0	1	4	6[b]
Bronchiolar alveolar Adenoma or Carcinoma	8	8	7	11	15
Bronchiolar alveolar Hyperplasia, Adenoma, or Carcinoma	10	17	15	16	18

[a] Data obtained from PWG report on page 2743 of study report (MRID No.49382174)
[b] Statistically significant by Cochran-Armitage trend test ($p < 0.05$)

The conclusion of the PWG was "The totality of the weight of evidence indicates that the slight difference in the incidence of bronchiolar alveolar carcinomas in the lungs of females at 7000 ppm, as compared to lower dose groups and the control group is a spurious finding and unrelated to the test article. The Pathology Working Group regarded this difference to represent normal biologic variation in the incidence of lung tumors in Crl:CD1®(ICR) mice and did not consider it to be indicative of a carcinogenic response." The PWG took into account a number of factors in arriving at their conclusion which included the following:

- There was no increase in precursor lesions (bronchiolar-alveolar hyperplasia or adenoma) that would be expected of a treatment-related effect.
- There was no evidence of pulmonary toxicity in male or female mice (e.g., inflammation, degeneration, or necrosis) in the study or in any earlier studies conducted in mice.
- There were no proliferative lesions in the lungs of male mice or in male or female rats from a 2-year chronic toxicity/carcinogenicity study.
- Lung tumors in CD-1 mice were considered a common neoplasm (>1.0% in controls) and occur with variable incidence.
- The increase in bronchiolar alveolar carcinoma was statistically significant only by Cochran Armitage trend test ($p<0.05$) (not by Fisher's Exact test) and would not be considered statistically significant when applying recently recommended statistical decision rules for common tumors ($p<0.005$ test for positive trend) (Lin and Rahman, 1998; Lin, 2000; FDA, 2001).
- Triflumezopyrim was not genotoxic in a battery of *in vitro* and *in vivo* studies.
- The results of investigative studies indicated a lack of metabolism of triflumezopyrim in mouse (and human) lung microsomes, the lack of activation of Cyp2F2 (a prominent cytochrome P450 in mouse lung that metabolizes a number of primary lung toxicants), and the lack of increased proliferation of the bronchiolar alveolar epithelium in female mouse lung following 3 or 7 days dietary exposure to triflumezopyrim

The PWG report on the lung tumors observed in female mice is included as Appendix L of the study report (MRID 49382174).

III. DISCUSSION AND CONCLUSION

A. INVESTIGATOR'S CONCLUSIONS:

The NOAEL was 2500 ppm (248.3 mg/kg bw/day) for males and 7000 ppm (809.8 mg/kg bw/day) for females. The LOAEL was based on anatomic pathology findings at 7000 ppm in males and a lack of adverse effects at 7000 ppm in females. Triflumezopyrim was not oncogenic at dietary concentrations up to and including 2500 ppm for males and up to and including 7000 ppm for females. Test-article related neoplastic findings were limited to benign hepatocellular adenoma in males only at the highest dietary concentration administered in this study, which was 7000 ppm.

B. REVIEWER'S COMMENTS:

No treatment-related effects were seen on survival, clinical signs, body weight, food consumption, and clinical pathology parameters at any dose level. Increases observed in absolute and relative liver weights were minimal and histopathology revealed hepatocellular hypertrophy in males at 2500 and 7000 ppm and in females at 7000 ppm.

The reviewers do not concur with the NOAEL/LOAEL established by the investigators for males. In the absence of other effects indicative of liver injury (e.g., alterations in relevant clinical chemistry parameters and/or histopathological findings), changes in liver weights and hypertrophy observed at 7000 ppm should be considered to be non-adverse, adaptive response to exposure to a xenobiotic.

The NOAEL in CD-1 mice was 7000 ppm, the highest concentration tested (726.72 mg/kg/day in males and 809.75 mg/kg/day in females). A LOAEL was not established.

Increased incidences of hepatocellular adenomas were observed in males and bronchiolo-alveolar carcinomas in females at 7000 ppm.

C. STUDY DEFICIENCIES:

None

D. REFERENCES

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